

# Identifying Loci for Behavioral Traits Using Genome-Tagged Mice

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Identification of behavioral loci through complex trait mapping remains a widely employed approach but suffers from poor gene localization and low replicability. Genome-tagged mice (GTMs) are overlapping sets of congenic strains spanning the whole genome and offer the possibilities of superior mapping power and reproducibility. In this study, three GTM strains each consisting of an average ~27 cM DBA/2J genomic intervals introgressed onto a C57BL/6J background were employed for localization of behavioral traits. These GTMs were chosen because the corresponding chromosomal regions had been previously identified as containing loci for learning and memory. Analysis of the GTMs allowed confirmation of the learning and memory loci, and one on chromosome 3 was in addition fine mapped to an 8.8-cM region of overlap between two of the GTMs. Moreover, loci for prepulse inhibition of the startle response, acoustic startle response, and spontaneous locomotor activity were also mapped. These results suggest that the GTMs should be a valuable resource for mapping and confirmation of loci contributing to complex behavioral traits in the mouse. © 2003 Wiley-Liss, Inc.

**Key words:** behavior; congenics; genetic mapping; learning and memory

The genetic basis of common behavioral traits is a problem of considerable scientific interest as well as being relevant to a better understanding of the neuropsychiatric disorders. Substantial efforts have been devoted to genetic mapping of behavioral phenotypes in the mouse, resulting in the identification of loci for learning and memory (Caldarone et al., 1997; Wehner et al., 1997), fear and anxiety (Flint et al., 1995; Gershensfeld et al., 1997; Gershensfeld and Paul, 1997; Talbot et al., 1999), sensorimotor gating (Geyer et al., 2002), and depression (Yoshikawa et al., 2002). Unfortunately, a number of factors complicate recovery of the responsible genes. These include the large numbers of loci that can play a role in behavioral traits, the allelic heterogeneity, the intricate interplay of epistasis and synergy, as well as the gene–environment interactions (Flint, 1999; Wehner et al., 2001). In addition, analysis of

behavior can suffer from problems of low replicability and high interindividual variation (Crabbe et al., 1999).

A potentially fruitful approach to the finer localization of genes contributing to complex behavioral traits is to develop and analyze congenic mouse strains for the associated critical regions. However, substantial investments are required to develop congenic strains for each genomic region of interest. An alternative strategy that has become feasible with the development of high-resolution genetic maps for the mouse is the use of marker-assisted breeding for rapid construction of congenic strains (“speed congenics”). This approach allows the creation of genome-wide libraries in which defined segments of the genome of one strain are propagated on the background of another. Genome-tagged mice (GTMs) represent such a resource, and two sets of these mice have been constructed. Both sets have C57BL/6J (B6) as the background strain, with one set having DBA/2J (D2) as the donor strain (B6.D2) and the other set having CAST/Ei (B6.CAST; Iakoubova et al., 2001). Each GTM set contains more than 60 strains, with on average a 23-cM introgressed donor segment (range 8–58 cM, 0.6–4% of the genome). These libraries represent a useful general resource for mapping genes involved in complex behavioral traits.

In this report, we describe the use of three GTM strains from the B6.D2 set to map genes for behavioral traits. The D2 and B6 parental strains are genetically distinguishable and have numerous phenotypic differences, including many in behavior (Crawley et al., 1997).

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The genomic regions investigated in this report were from the proximal region of chromosome 3 (3P; ~2.2 cM to ~29.5 cM), the middle region of chromosome 3 (3M; ~29.5 to ~57.9 cM), and the proximal region of chromosome 10 (10P; ~0 cM to 25.1 cM). These regions were chosen for analysis because they had been implicated in learning and memory based on complex trait mapping experiments using context- and cue-dependent fear conditioning (Caldarone et al., 1997; Wehner et al., 1997). In addition to fear conditioning, the GTMs were also assessed for the following behaviors: sensorimotor gating, acoustic startle response, fear and anxiety, spontaneous locomotor activity, behavioral despair, and pain sensitivity. By using the GTMs, loci for acoustic startle response, spontaneous locomotor activity, and learning and memory were mapped to both chromosomes 3 and 10, and an additional locus for sensorimotor gating was found on chromosome 3 but not chromosome 10.

## MATERIALS AND METHODS

### Mice

Male mice of age 8–11 weeks were tested. The mice were group housed. The GTMs were BDChr3P, 3M, and 10P (Iakoubova et al., 2001), in which the B6 background strain is replaced with the following segments from the D2 genome: 3P, proximal chromosome 3 from ~2.2 cM to ~29.5 cM (proximal uncertainty: 0 cM, D3Mit360, to 2.2 cM, D3Mit149; distal uncertainty: 29.5 cM, D3Mit97, to 33.9 cM, D3Mit29); 3M, middle chromosome 3 from ~29.5 cM to ~57.9 cM, (proximal uncertainty: 25.1 cM, D3Mit97, to 29.5 cM, D3Mit97; distal uncertainty: 57.9 cM, D3Mit45, to 63.4 cM, D3Mit128); 10P, proximal chromosome 10 from ~0 cM to ~25.1 cM (proximal uncertainty: 0 cM, D10Mit305; distal uncertainty: 25.1 cM, D10Mit15, to 33.9 cM, D10Mit198). Note that the maximal overlap between 3P and 3M is 8.8 cM (extending from 25.1 cM, D3Mit22, to 33.9 cM, D3Mit29), and the minimum overlap is 0 cM (extending from 29.5 cM, D3Mit97, to 29.5 cM, D3Mit97). The parental control strains, D2 and B6, were purchased from Jackson Laboratories (Bar Harbor, ME). The following numbers of mice were employed: 3P,  $n = 22$ ; 3M,  $n = 19$ ; 10P,  $n = 19$ ; B6,  $n = 20$ ; D2,  $n = 20$ .

### Behavioral Testing

Animals were housed on a 12-hr/12-hr light/dark cycle. The light phase was from 6 AM to 6 PM, and tests were conducted between 10 AM and 4 PM. Testing equipment was cleaned with 1:32 bleach solution between animals. All mice were assessed using behavioral tests in the following order: open field, spontaneous locomotor activity, prepulse inhibition of the startle response, tail flick, Porsolt forced swim test, and context- and cue-dependent fear conditioning.

### Prepulse Inhibition

Prepulse inhibition (PPI) of the acoustic startle response (ASR) was used to assess sensorimotor gating (Paylor and Crawley, 1997; Sayah et al., 2000). The Startle Reflex Lab controlled by SR-Lab software (San Diego Instruments, San Diego, CA) was employed for this test. A mouse was confined to a cylindrical

holder inside a sound-attenuating chamber, and background noise was set to 65 dB. Pulse-alone trials (P) consisted of a single white-noise burst (120 dB, 40 msec). The prepulse + pulse trials (PP74P, PP77P, PP80P) consisted of a prepulse of white noise (20 msec at 74 dB, 77 dB, or 80 dB, respectively), followed 100 msec after onset of prepulse by a white-noise pulse (120 dB, 40 msec). No-stimulus (NS) trials consisted of background noise only. Data in pulse and prepulse trials was recorded at the onset of the 120-dB pulse for 65 msec in 1-msec increments. Sessions were structured as follows: 1) five minute acclimation at background noise level, 2) five P trials, 3) ten blocks each containing all five trials (P, PP74P, PP77P, PP80P, NS) in pseudorandom order, 4) five P trials. Intertrial intervals were pseudorandomly distributed between 10 and 20 sec. The maximum force intensity of startle for each trial ( $V_{max}$ ) was recorded using a piezoelectric transducer in the floor of the cylindrical holder. The average percentage reduction in startle intensity between pulse and prepulse + pulse trials at all three prepulse levels was defined as the PPI level.

### Tail Flick

The tail-flick assay was used to assess pain sensitivity (Sayah et al., 2000). The mouse being tested was loosely restrained by hand in a paper towel on a Columbus Instruments Tail-Flick Apparatus. The tail of the animal was allowed to rest freely in a groove in the apparatus above a shuttered lamp. A foot trigger opened the shutter and started a timer. The heat from the lamp provided a nociceptive stimulus eventually causing the mouse to flick its tail away from the groove. The tail-flick latency provided a measure of the animal's perception of pain. The lamp intensity was set at 8 of 25, and the subject's latency was determined by the instrument's autodetection capability, which was based on interruption of an infrared beam by the tail flick. For each mouse, the tail-flick value was taken as the average of three trials.

### Open Field

The open-field test was used to quantitate fear and anxiety (Flint et al., 1995; Gershenfeld et al., 1997; Gershenfeld and Paul, 1997; Talbot et al., 1999; Sayah et al., 2000). A 1-m-diameter circular drum with 40-cm walls and pressed-wood floor was employed. The interior of the drum was painted white, and lights were mounted directly above. The large, brightly lit arena is considered an anxiety-provoking environment. Mice were tracked using the Poly-Track computerized video tracking system controlled by Chromo-Track software (San Diego Instruments). The peripheral region was defined as the area outside a circle of diameter 0.67 m. Data recording began with the mouse placed in the center of the arena and was continued for 5 min. Latency to enter the peripheral region was taken as a measure of fear and anxiety. An anxious mouse will immediately scurry to the periphery, registering a low latency to leave the center, whereas a bold mouse will explore more freely.

### Fear Conditioning

Context- and cue-dependent fear conditioning was used to assess learning and memory (Fanselow and Bolles, 1979; Sayah et al., 2000; Xie et al., 2000). This test quantitates a mouse's ability to remember the context in which it had pre-

viously received a mild electric foot shock. The fear-conditioning chamber consisted of one side of a Gemini Avoidance System (San Diego Instruments). Access to the other side of the apparatus was blocked. Conditioning trials were structured as follows: the mouse was placed in the chamber, and after 2 min a tone (2,900 Hz at 8.5 dB) was activated for 30 sec. This tone is the conditioned stimulus (CS). The last 2 sec of the tone were paired with a foot shock (0.70 mA), and the animal was allowed an additional 30 sec in the chamber before being removed to its home cage. Freezing behavior (defined as absence of all but respiratory movements) was assessed every 5 sec over consecutive 30-sec blocks for the 3-min training period by an observer who entered data directly into a computer spreadsheet program in real time.

In the context-dependent phase 24 hr later, the mouse was returned to the conditioning chamber. Freezing was assessed in the same manner as employed for the training period. The percentage of time the animal spent frozen was taken as a measure of fear and thus the ability to remember the previous day's conditioning trial. This phase of the test requires both a correctly functioning hippocampus and amygdala. Cue-dependent conditioning was performed by placing the mouse in a novel environment, a 20-cm-diameter, 50-cm-high cylindrical beaker, between 30 min and 1 hr after the context testing phase. The animal was allowed to acclimatize for 3 min in the novel environment and then exposed to the CS for 3 min. Freezing was measured as before, except blocks of 1 min were employed for the 6-min testing period. Freezing on this phase of the test is evidence for a normal fear response and requires a functioning amygdala, but not hippocampus. To control for pain sensitivity, threshold currents for the following responses were measured, in order of increasing aversion: flinch, jump/run, and vocalization.

### Spontaneous Locomotor Activity

Locomotor activity was measured for 1 hr in the dark using the Cage Rack System (San Diego Instruments). The cages were 47 × 26 × 15 cm and had a uniformly spaced, 8 × 4 photobeam grid. Locomotor activity was measured by counting the total number of beam breaks during the testing period.

### Porsolt Forced-Swim Test

The Porsolt test is a commonly used test of behavioral despair or depressive-like behaviors (Porsolt et al., 1977). Anti-depressant drugs increase the percentage of time spent swimming or climbing in this test. A mouse was placed in a plastic cylinder of diameter 25 cm, height 65 cm, containing water at a temperature of 22–24°C and a depth of 46 cm so that it could neither escape nor touch the bottom. The animals were tested for 5 min. The presence of swimming or climbing was quantitated using 5-sec blocks and recorded in real time using a computer spreadsheet.

### Statistical Analysis

Statistical significance was assessed using analysis of variance (ANOVA), with Dunnett's correction for multiple comparisons to the control B6 parental strain. A probability level of  $P < .05$  was used as the threshold for statistical significance.

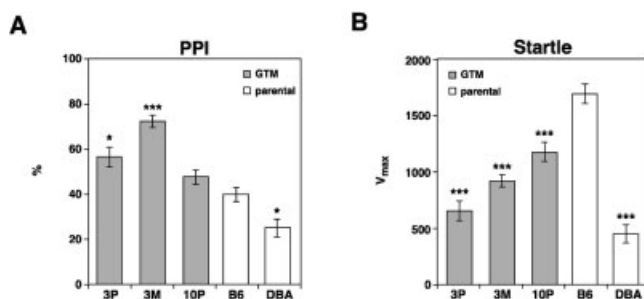


Fig. 1. PPI and ASR. **A:** PPI is expressed as percentage reduction in startle from average startle response across the three prepulse trials employed. **B:** Acoustic startle response (ASR).  $V_{max}$  is the maximum force intensity for each trial. \* $P < .05$ , \*\*\* $P < .0001$  compared with B6, Dunnett's test.

## RESULTS

### Prepulse Inhibition

The PPI was used to assess sensorimotor gating, an attentional mechanism. Both 3P and 3M showed significantly greater PPI than the B6 parental background strain (Fig. 1A). This difference implies the presence of a locus for PPI on chromosome 3. There is probable overlap between the 3P and 3M GTMs (up to 8.8 cM, extending from 25.1 cM to 33.9 cM), so this observation either suggests the presence of a single locus responsible for PPI on chromosome 3 in the region of overlap or the presence of two separate loci, one on 3P and one on 3M. There was no locus for PPI apparent in the tested proximal region of chromosome 10.

All three GTMs tested displayed significantly decreased levels of acoustic startle response (ASR) in the absence of a prepulse compared with the parental B6 background strain (Fig. 1B). This suggests the presence of at least one and possibly two loci for ASR on chromosome 3 and also the presence of a locus for ASR on chromosome 10. The B6 strain showed about a fourfold higher startle response than the D2 strain, also consistent with published reports (Paylor and Crawley, 1997). Some of the differences in ASR between the D2 and B6 parental strains may be explained by the fact that D2 shows progressive hearing loss beginning before 3 months of age, whereas B6 shows late-onset hearing loss beginning after 10 months of age (Zheng et al., 1999). This hearing loss is at least partially accounted for by a recessive locus, *age related hearing loss (Ahl)*, which maps to 23 cM on chromosome 10 (Johnson et al., 2000). The *Ahl* locus is encompassed by 10P (proximal uncertainty: 0 cM; distal uncertainty: 25.1 cM to 33.9 cM) but is unlikely to account for the significant ASR results of 10P compared with B6, insofar as this locus is allelic in D2 and B6. The later onset of age-related hearing loss in B6 compared with D2 is due to modifiers extragenic to *Ahl*, which implies that the 10P GTM should have a late onset of hearing loss consistent with the B6 background present in the tested GTMs. This is contrary to the decreased ASR of the 10P GTM. In addition,

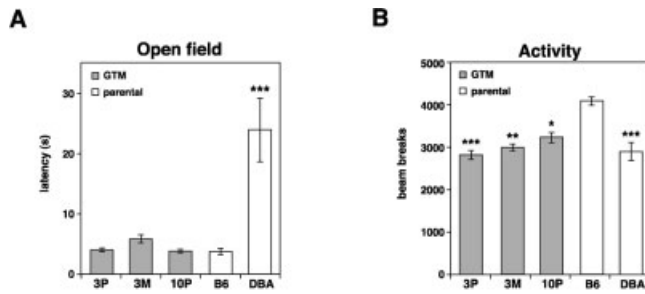


Fig. 2. Open-field test and spontaneous locomotor activity. **A:** Open-field test. Latency to reach outer circle of the open field is shown. **B:** Spontaneous locomotor activity was measured as total beam breaks. **\*\*** $P < .01$ , Dunnett's test.

the hearing loss of D2 and B6 is greatest for pure high tones and becomes less of a concern when broad-band white noise is used, as is the case for PPI and ASR (Geyer et al., 2002). Hearing loss is also minimized by the use of young adult mice, and the animals employed for this study were 8–11 weeks of age.

A recent study of PPI and acoustic startle using recombinant inbred mouse strains found a potential locus for PPI and ASR in the middle of chromosome 3 (~33.70 cM to 66.20 cM; Joobert et al., 2002) consistent with the findings described here. However, this study did not find any loci involved with PPI or ASR on chromosome 10. Part of the reason for the discrepancy might be the disparity in parental strains for the recombinant inbred study (B6 and A/J) vs. the present study (B6 and D2). In addition, the A/J strain features early-onset hearing loss dependent on *Ahl*, similar to D2 (Johnson et al., 2000). The fact that study of the recombinant inbreds did not identify a locus for ASR on proximal chromosome 10 further suggests that the locus for ASR identified here using 10P is not due simply to early-onset hearing loss.

### Open Field

In the open-field test, mice with high fear and anxiety show low latency to move to the periphery of a brightly lit drum, a potentially less threatening region of the open field. The open-field test revealed no significant differences for all three of the GTMs compared with the parental B6 strain (Fig. 2A), suggesting the absence of loci for fear and anxiety in the tested regions of chromosome 3 and also chromosome 10. Consistently with this result, complex trait mapping revealed no loci for fear and anxiety in these regions of the genome (Flint et al., 1995; Gershenfeld et al., 1997; Gershenfeld and Paul, 1997; Talbot et al., 1999).

### Spontaneous Locomotor Activity

There were significant differences for all three GTMs compared with the B6 parental strain (Fig. 2B), suggesting the possibility of loci for spontaneous locomotor activity on both chromosome 3 and chromosome 10. These results are consistent with data obtained using re-

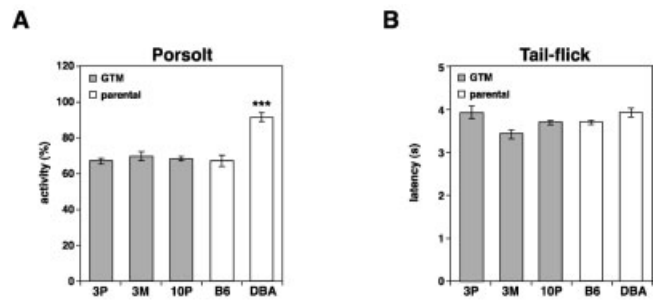


Fig. 3. Porsolt forced-swim and tail-flick tests. **A:** Porsolt forced-swim test. The percentage of time spent swimming or climbing is shown. **B:** Tail-flick test. Latency shown in seconds.

combinant inbred strains, which also found loci for locomotor activity on these chromosomes (Toth and Williams, 1999; Koyner et al., 2000).

### Porsolt Swim Test

This test of depressive-like behavior quantitates the percentage of time a mouse spends trying to exit from a water-filled beaker from which escape is impossible. Antidepressants increase the percentage of activity time on this test. The GTMs showed no significant difference compared with the B6 strain (Fig. 3A), suggesting the absence of a locus for behavioral despair in the tested regions of the genome. This is consistent with a recent study that used complex trait mapping to identify loci involved in the Porsolt forced-swim test (Yoshikawa et al., 2002). No loci were found for this trait on chromosome 3 or 10.

### Tail Flick

There was no significant difference between the GTMs and the B6 parental strain in this test of nociception, indicating the absence of a locus for pain sensitivity in the tested chromosomal regions (Fig. 3B).

### Context- and Cue-Dependent Fear Conditioning

Context- and cue-dependent fear conditioning is a widely employed test of learning and memory (Fanselow and Bolles, 1979). This test was used to confirm potential loci for learning and memory in the GTMs. In the training phase of the test (Fig. 4A), mice receive an auditory tone (CS) and coincident electrical foot shock. The training phase is hence divided into two epochs, before conditioned stimulus (BC) and after conditioned stimulus (AC). In the BC epoch, the 3P strain showed significantly greater freezing than the B6 parental strain. However, the remaining GTMs showed no significant differences. Comparing the AC epoch with the BC epoch, a significant increase in freezing was found for all strains, both GTM and parental ( $P < .0001$ ). Furthermore, in the AC epoch, the 10P GTM strain showed significantly greater freezing than B6.

In the context-dependent part of the test, 24 hr later, mice were placed back in the chamber in which they had

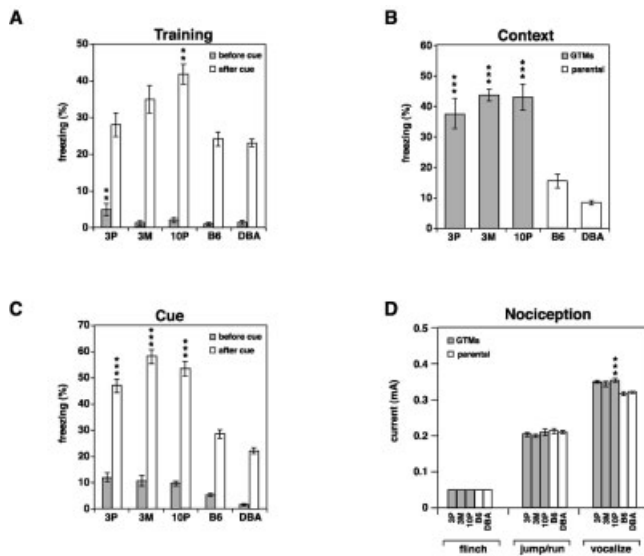


Fig. 4. Context- and cue-dependent fear conditioning. **A:** Training. **B:** Context-dependent conditioning. **C:** Cue-dependent conditioning. **D:** Nociception.

previously received the electric shock. Proper freezing in this phase of the test requires correct learning and memory to allow the mice to recognize the context in which they had received the foot shock, so a correctly functioning hippocampus is required. In addition, an appropriate fear response is necessary, which depends on a correctly functioning amygdala. In this phase (Fig. 4B), all three GTMs showed significantly greater freezing than either of the parental strains, suggesting at least one locus for learning and memory on chromosome 3 and one on 10P. There was no significant difference in freezing between the parental strains. However, all strains showed significantly greater freezing when we compared the first 2 min of the context phase of the test with the BC epoch of the training phase ( $P < .0001$ , all cases). This observation suggests that both parental strains (as well as the GTM) had learned the context in which the shock had been administered, despite the fact that the level of freezing for the parental strain was much less than that shown by the GTMs.

The cue-dependent phase of the test is a control to ensure an effective fear response, a function of the amygdala but not the hippocampus. In this phase of the test, all strains analyzed showed significantly greater freezing AC compared with BC ( $P < .0001$ ; Fig. 4C), suggesting correct fear responses for all GTM and parental strains. However, there were significant differences in the magnitude of the freezing response in the AC epoch, with all three GTMs showing significantly greater freezing than the parental strains, similar to what was seen in the context part of the test (Fig. 4B).

An important control for context- and cue-dependent fear conditioning is sensitivity to the electric foot shock (Fig. 4D). The only difference between the three strains was at the highest response, vocalization,

where the 10P GTM showed significantly greater current thresholds than B6.

The data indicate dramatically higher levels of performance on both the context- and the cue-dependent phases of the test for the GTMs compared with the parental strains. These differences cannot be explained by altered pain sensitivity of the GTMs; their sensitivity, if anything, is less than that of the D2 and B6 parentals, as indicated by the significantly higher current threshold of 10P for vocalization (Fig. 4D). It is also unlikely that age-related hearing losses of D2 and B6 (Zheng et al., 1999) contributed to the differences in the cue-dependent fear conditioning, in that the GTM and parental strains showed highly significant increases in freezing as a result of the CS in the cue-dependent part of the test ( $P < .0001$ ). Furthermore, young adult mice, 8–10 weeks of age, were employed in this study, minimizing the effect of any hearing loss.

The context-dependent data suggest the presence of a strong locus for learning and memory in the tested regions of chromosomes 3 and 10, whereas the cue-dependent conditioning suggests loci for fear. These findings are in agreement with results from complex trait mapping (Caldarone et al., 1997; Wehner et al., 1997), which found loci for both context- and cue-dependent conditioning in the relevant chromosomal regions.

## DISCUSSION

Because of the relatively poor mapping power of complex trait analysis, complementary strategies must be employed for fine localization of genes that play a role in behavior. The results reported here suggest that the GTMs will be a valuable resource for fine mapping of such traits. Three GTMs were chosen for analysis because they covered regions that had previously been implicated in learning and memory through complex trait mapping. In addition to learning and memory, phenotypes related to sensorimotor gating, startle, fear and anxiety, locomotor activity, behavioral despair, and pain sensitivity were also assessed. Loci on chromosomes 3P, 3M, and 10P were found for ASR, spontaneous locomotor activity, and learning and memory. For chromosomes 3P and 3M but not 10P, a locus was also found for PPI.

The most parsimonious explanation of the PPI data suggests a single locus for PPI and ASR mapping to an 8.8-cM region of chromosome 3, ranging from 25.1 cM to 33.9 cM. Loci for PPI and ASR have also been mapped using recombinant inbred mice derived from B6 and A/J parental strains (Joober et al., 2002). Consistently with the data reported here, the Joober et al. study mapped a locus for PPI to a region of chromosome 3 ranging from ~33.7 cM to 66.2 cM. However, in contrast to the findings of the present study, the recombinant inbred investigation found no locus for ASR on chromosome 10P. This discrepancy may be due to the use of different parental strains in the two investigations. In addition, the effect of the 10P locus uncovered in the present investigation was the weakest of the three GTMs tested and might not be

detectable in an experiment using recombinant inbreds with multiple introgressed genome segments.

Similarly to the PPI data, loci for spontaneous locomotor activity were found on chromosomes 3 and 10. Loci have also been identified for this behavior on chromosomes 3 and 10 using recombinant inbred mouse strains (Koyner et al., 2000; Toth and Williams, 1999).

Analysis of the GTMs revealed loci for context- and cue-dependent fear conditioning on chromosomes 3P, 3M, and 10P. This result is consistent with published reports from complex trait mapping, which identified loci for fear conditioning in the central region of chromosome 3 and the proximal/central region of chromosome 10 (Caldarone et al., 1997; Wehner et al., 1997). Note that, if a single locus on chromosome 3 is responsible for fear conditioning, the data reported here have narrowed the region responsible for this behavior to 8.8 cM. The GTMs showed performance on context- and cue-dependent fear conditioning that was superior to that of either of the parental strains. This pattern of phenotypic expression is not unexpected and reflects the intricate genetic relationships that can occur between alleles in complex traits. For example, the complex trait mapping studies of fear conditioning showed increased performance for the D2 alleles of a chromosome 1 locus, in spite of the fact that B6 showed greater freezing than D2 (Caldarone et al., 1997; Wehner et al., 1997).

It is unlikely that the differences in learning and memory between the GTM and the B6 are due to non-specific effects of the introgressed D2 segment. This is because the GTMs showed no significant difference from the B6 background strain for many of the behaviors analyzed, including fear and anxiety (open field), depressive-like behaviors (Porsolt forced swim), and nociception (tail flick).

A possible caveat to the conclusions presented here is that the employed GTM strains have segregating regions of unwanted heterozygosity from other chromosomes (Iakoubova et al., 2001). The 3P GTM has additional fragments from D2 chromosome 1; 3M has additional fragments from chromosomes 5, 13, and 15; and 10P has additional fragments from chromosomes 9, 13, and 15. However, for the chromosome 3 GTMs, this potential confound is all but eliminated by the fact that there is no additional D2 genetic material common to both 3P and 3M. Study of further chromosome 10 GTMs would eliminate the concern of unwanted heterozygosity for this chromosome, while also providing finer localization of potential behavioral loci.

A map of the chromosome 3 and 10 regions tested using the GTMs in this study is shown in Figure 5. Also shown are the underlying lod plots for context- and cue-dependent fear conditioning (Caldarone et al., 1997; Wehner et al., 1997). For both chromosome 3 and chromosome 10, it can be seen that the tested GTMs overlay the lod peaks for fear conditioning. For chromosome 3, in particular, the 8.8-cM critical region of overlap between

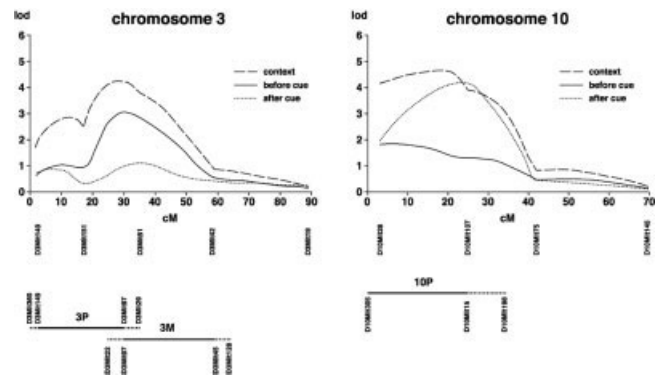


Fig. 5. Map of the chromosome 3 and 10 regions. The underlying lod plots for context- and cue-dependent fear conditioning are shown (Caldarone et al., 1997; Wehner et al., 1997). The proximal and distal limits of uncertainty for the GTMs are depicted as dotted lines together with the relevant markers.

3P and 3M neatly corresponds to the fear-conditioning lod peak.

There are approximately 150 known genes in this critical region (UCSC Genome Browser: genome.ucsc.edu). A few potential candidate genes for the learning and memory locus are shown in Figure 6. Butyrylcholinesterase (Bche) is related to acetylcholinesterase and may have important roles in cholinergic neurotransmission (Darvesh et al., 2003). Inhibition of the Bche gene product is a potential strategy for treatment of Alzheimer's disease. Glutamate receptor 2 (Gria2, GluR2, GluR-B) null mutant mice show enhanced non-Hebbian long-term potentiation (LTP) in hippocampal CA1 neurons, an *in vitro* model of learning and memory (Jia et al., 1996; Yan et al., 2002). In addition, these animals show possible impaired performance in a spatial learning task. Mutations in the  $\beta$ -subunit of the inhibitory glycine receptor (Glr $\beta$ ) result in both murine and human hyperekplexia, a neurological disorder characterized by an excessive startle response (Rees et al., 2002).

The neuropeptide Y  $Y_2$  receptor (Npy2r) is the predominant neuropeptide Y receptor subtype in the brain and is found in specific subregions of the hippocampus and hypothalamus (Kaga et al., 2001). It has been demonstrated in rats that the ability of NPY to enhance learning and memory is linked mainly to activation of the  $Y_2$  receptor subtype. Secreted frizzled-related sequence protein 2 (Sfrp2) is a Wnt antagonist that stimulates production of neural progenitors (Aubert et al., 2002) and is expressed in the presumptive forebrain and neural tube (Pera and De Robertis, 2000). Nestin (Nes) is an intermediate filament protein expressed in central nervous system stem cells (Cunningham and McKay, 1994). Brevican (Bcan) is a brain-specific proteoglycan, absence of which results in deficits in maintenance of hippocampal LTP in knockout mice (Brakebusch et al., 2002). However, these abnormalities are not associated with any observable learning and memory deficits.

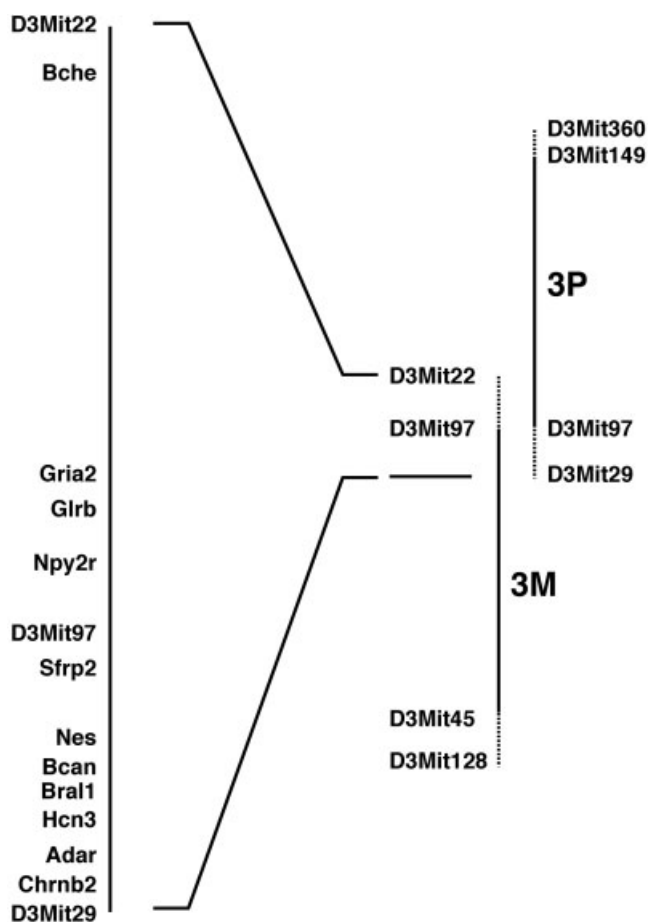


Fig. 6. Potential candidate genes for the learning and memory locus in the 8.8-cM critical region of chromosome 3. *Bche*, butyrylcholinesterase; *Gria2*, glutamate receptor 2 (GluR2, GluR-B); *GlrB*,  $\beta$ -subunit of the inhibitory glycine receptor; *Npy2r*, neuropeptide Y  $Y_2$  receptor; *Sfrp2*, secreted frizzled-related sequence protein 2; *Nes*, nestin; *Bcan*, brevicin; *Bral1*, brain link protein-1; *Hcn3*, hyperpolarization-activated cyclic-nucleotide-gated cation channel HAC3; *Adar*, adenosine deaminase 1; *Chrnb2*, nicotinic acetylcholine receptor  $\beta_2$  subunit.

Brain link protein-1 (*Bral1*) is found in myelinated fiber tracts in the adult brain and is localized to the nodes of Ranvier (Oohashi et al., 2002). Hyperpolarization-activated cyclic nucleotide-gated cation channel HAC3 (*Hcn3*) is expressed at a low level throughout the brain (Moosmang et al., 1999). Adenosine deaminase 1 (*Adar*) is a mammalian RNA editing enzyme that plays a role in editing of glutamate receptor 2 (*Gria2*, GluR2, GluR-B) mRNA. Interestingly, the editing substrate for this gene is also found in the 8.8-cM critical region (see above). Absence of the nicotinic acetylcholine receptor  $\beta_2$  subunit (*Chrnb2*) in knockout mice results in deficits in spatial learning in aged animals (Zoli et al., 1999; Caldarone et al., 2000).

Further localization of the gene responsible for the behavioral phenotypes in the 8.8-cM critical region could be accomplished using additional marker assisted breeding

or by phenotyping and mapping of F2 intercross animals resulting from 3P and 3M parentals. If the responsible gene displays differential expression in the hippocampi of 3P and 3M mice, transcript profiling may also be a fruitful approach. This strategy has been employed for gene identification for other traits (Aitman et al., 1999).

An additional GTM from the middle region of chromosome 1 has been analyzed using the battery of tests described here (Liu et al., 2003). This GTM was found to harbor a strong locus for learning and memory as assessed by using fear conditioning, replicating the results from complex trait mapping. However, no loci were found for other behaviors, except a minor effect locus for spontaneous locomotor activity. In combination, then, the results from the GTMs replicate three major loci for learning and memory identified using complex trait mapping on chromosomes 1, 3, and 10 (Caldarone et al., 1997; Wehner et al., 1997). Furthermore, the GTM data potentially narrow down the locus on chromosome 3 to an interval of 8.8 cM, greatly facilitating further attempts at positional cloning of this locus. Thus, the results strengthen the idea that the genome-wide and overlapping characteristics of the GTMs should allow them to be a useful reagent for fine mapping and genetic dissection of complex behavioral traits in the mouse.

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